

ORIGINAL RESEARCH

## Enhancing Soil Fertility of Apple Orchard through Biochar and Fertilizer Amendments: A Soil Aggregation Study

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**ABSTRACT:** The declining productivity of apple trees can be attributed to the adverse effects of unbalanced climatic conditions and dynamic soil properties. Addressing these challenges through sustainable agricultural practices is crucial to improving apple orchard productivity and ensuring a resilient agricultural system. To enhance the function of fragile ecosystem services, the addition of biochar at an appropriate rate along with chemical fertilizers (NPK) is considered an efficient approach for improving apple trees productivity. The treatments combinations were 0 t ha<sup>-1</sup> (CK), 4 t ha<sup>-1</sup> (T1), 8 t ha<sup>-1</sup> (T2), 12 t ha<sup>-1</sup> (T3), 16 t ha<sup>-1</sup> (T4), and 20 t ha<sup>-1</sup> (T5). Our results demonstrated that, biochar addition rate in the T5 significantly increased macro-aggregates (WSAs > 0.25 mm), mean weight diameter (MWD) and therefore decreased micro-aggregates (WSAs < 0.25 mm) compare to the control. Soil organic carbon (SOC) and total nitrogen (T.N) in both the bulk soil and water stable aggregates (WSAs) showed similar and an increased trend with biochar addition rate. However, the trend of C:N ratio was in opposition with biochar addition rate for both the bulk soil and WSAs. Additionally, biochar addition rate (T5) significantly intensified partitioning proportion (%) of the SOC, and T.N in WSAs > 0.25 mm, and WSAs < 0.25 mm and therefore showed non significance differences for the others treatments. Such a partitioning proportion of the WSAs 0.5-0.25 mm were lower than the WSAs > 0.5 mm and WSAs < 0.25 mm. These results suggested that biochar addition rate (T5) with chemical fertilizer had a significant effect on the stability of aggregates associated SOC, T.N, and C:N ratio and it may also have a capability in optimizing partitioning proportion (%) of the SOC and T.N in WSAs > 0.25 mm. Thus, it is therefore suggested that biochar addition rate (T5) with chemical fertilizers is the best preference for the stability and optimization of the aggregate associated SOC and T.N which may enhance partitioning proportion (%) of the SOC and T.N in an apple growing soil.

**KEYWORDS:** Biochar, Apple orchards, Water stable aggregates, Soil organic carbon, Total nitrogen

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### 1. Introduction

Apple (*Malus domestica* borkh) is one of the most essential temperate crops in Asia and Europe. Apple belongs to the *Rosaceae* family, and it is the most used and widely cultivated fruit (Ullah et al., 2021; Zhang et al., 2023). Pakistan grows a variety of apples

including Mashaday, Kashmiri, Amri, Sky Spur, Kala Kulu, Red Delicious and Golden Delicious (Mukhtar et al., 2010). Notably, the region boasts a rich tradition of cultivating globally significant fruits, particularly apples fourth in number in Pakistan after citrus, mango and banana, originating from

Southwestern Asia and flourishing in the hilly terrains of Punjab, Khyber Pakhtunkhwa, and Balochistan within Pakistan (Shah et al., 2016). Agriculture 2014-2015, Apple fruits were yielded on 100246 hectares of land and its production in Pakistan was 616748 tons, while in KP, apple has been cultivating under in an area of 7983 hectares yielding a production of 97619 tons. Specially in Swat, the reported production stands at 32,000 tons within a cultivated area of 3,750 hectares (AMIS, 2015), is the most important of all the apple-growing districts in KP, followed by Dir, Mansehra, Chitral and Abbottabad districts (Bokhari, 2002). The Swat district comprises Lower Swat and Upper Swat; the climate in Upper Swat experiences more severe winters compared to the Lower Swat region. This valley is renowned for producing high-quality fruits, including peaches, apricots, apples, walnuts, and plums (Ullah et al., 2021; Ali .S, 2023). However, a concerning trend looms over the Swat district the dwindling productivity of apple orchards. Soil fertility issues and the impact of shifting climatic conditions have been identified as primary culprits (Shah et al., 2016). However, a limited number study has been conducted on apple in Pakistan, which is limited to northern hilly areas of Punjab, KP and Baluchistan (Mukhtar et al., 2010). In response to this challenge, our study takes center stage, aiming to reverse the declining trend by investigating the potential benefits of biochar and fertilizer applications in enhancing apple orchard productivity. As we navigate through the intricacies of this research, we aspire to shed light on viable solutions that not only address the current challenges faced by apple

orchards in Swat but also pave the way for a more resilient and productive future. The promising prospect of biochar and fertilizer applications holds the key to unlocking new possibilities for apple production in the Swat district, ensuring sustainable agricultural practices in the face of evolving environmental dynamics.

Soil aggregates is regarded as one of the key component in soil system influence biogeochemical processes of the soil (Mueller et al., 2007). Several studies reveal that stability of aggregates enhance water availability in alleviating soil carbon, and nitrogen losses (Shaver et al., 2002). However, external environment is the basic factor influence both the distribution, and development of soil aggregates (Mueller et al., 2007). Among the external environment, mechanical forces and rainfall are the two basic factor that disrupts aggregate stability in changing soil properties alter decomposition of the soil organic matter, carbon sequestration, nitrogen mineralization, and nutrient cycling (Rampazzo et al., 1995), (Six et al., 2000). These soil properties act as binding agents for the soil aggregation (Ali et al., 2022c; Ali et al., 2022a; Ullah et al., 2021). However, such a lower soil organic matter, and dense soil have stronger aggregates that is resistance to disruptions under dry conditions and such a resistance are weaken in wet conditions. Therefore, aggregates stability is one of the basic indexes for the field soil to avoid the risks of soil erosion with improved nutrients availability. Previous studies revealed that organic amendment is an important management practices in enhancing aggregates stability (Doan et al., 2014),.

Among the organic amendment, biochar is one of the carbonaceous material produced under anaerobic condition at high pyrolysis temperature (Ali et al., 2020b, Laird et al., 2009). Biochar has gained wide acknowledgement for more than a decade (Ali et al., 2021; Song et al., 2022; Arthur et al., 2015) in its usage for carbon sequestration and improving physicochemical properties of soil (Alghamdi, 2018), optimize soil quality (Woods et al., 2006), in reducing bulk density with enhancing soil porosity and aggregation (Ali et al., 2022a; Blanco-Canqui, 2017). Biochar modify soil acidity (Saha et al., 2020), and cation exchange capacity (Ghezzehei et al., 2014) may results in enhancing the abundance and diversity of microbes in the rhizosphere (Saha et al., 2020; Ali et al., 2022b). Due to considerable and valuable role of biochar on soil properties, the prospect of enhancing and stabilizing aggregates lower soil erosion therefore gained the interests of researchers. Researchers have extensively studied the effects of biochar on soil fertility, but its specific impact on ecosystem services related to soil aggregate size, organic carbon, and nitrogen remains unclear. Thus, the objective of this study was: (1) to assess the influence of biochar on the distribution and stability of aggregates associated with soil organic carbon (SOC), total nitrogen (T.N), and carbon-to-nitrogen (C:N) ratio, and (2) to investigate the proportion (%) of SOC and T.N partitioned within the water-stable aggregates (WSAs). The findings from this study have the potential to enhance the quality and productivity of apple orchards.

## 2. Materials and methods

### 2.1 Study site

Our research was carried out in the upper district of Swat, situated in Khyber Pakhtunkhwa (KP), Pakistan during 2022. In this region, the soil exhibits a slightly acidic to alkaline pH range, spanning from 7.21 to 8.27. Furthermore, the electrical conductivity (EC) values, ranging from 0.06 to 0.620 dSm<sup>-1</sup>, indicate that the soil is non-saline, with values well below the critical threshold of 4 dSm<sup>-1</sup>. As for the soil texture, it predominantly falls into the categories of silt loam and loamy sand. Notably, the soil in our study area exhibits a moderate level of calcareous content, which is considered advantageous for apple production due to its positive influence on nutrient availability and root health (Salam, et al. 2022). Biochar consisted of wood biomass that were subjected to pyrolysis at a temperature of 750 °C under anaerobic condition. The basic physicochemical properties of experimental field and biochar is given in Table 1

Table 1. Basic physico-chemical properties of experimental materials.

| Items  | Soil      | Biochar |
|--|-----------|---------|
| Sand (%)                                       | 26.28     | —       |
| Silt (%)                                       | 58.1      | —       |
| Clay (%)                                       | 15.24     | —       |
| Soil texture                                   | Silt loam | —       |
| Surface area (m <sup>2</sup> g <sup>-1</sup> ) | —         | 1.15    |
| SOC ( g kg <sup>-1</sup> )                     | 4.35      | —       |
| pH   | 7.36      | 8.5     |
| TN (g kg <sup>-1</sup> )                       | 0.55      | 5.42    |
| TP ( g kg <sup>-1</sup> )                      | 0.97      | 45.22   |
| TK( g kg <sup>-1</sup> )                       | —         | 46.35   |

Note: SOC—soil organic carbon, TN—total nitrogen, TP—total phosphorous, TK—total potassium.

## 2.2 Experimental set up and design

The experiment was conducted on randomized complete block design. The plots size was  $6 \times 4 = 24 \text{ m}^2$ . All plots received an equal amount of chemical fertilizer. Biochar addition rate were  $0 \text{ t ha}^{-1}$  (CK),  $4 \text{ t ha}^{-1}$  (T1),  $8 \text{ t ha}^{-1}$  (T2),  $12 \text{ t ha}^{-1}$  (T3),  $16 \text{ t ha}^{-1}$  (T4), and  $20 \text{ t ha}^{-1}$  (T5).

### 2.3 Soil sampling and analysis

During each treatment, top soil layer (0-20 cm) soil was taken for the analysis of physico-chemical properties of soil. Meanwhile, undisturbed soil samples for the analysis of aggregate stability were taken in triplicates in stainless steel boxes (20 cm x 12.5 cm x 6 cm). The stainless-steel boxes were sealed and kept in polyethylene bags, and were brought into the laboratory for the analysis of water stable aggregates.

### 2.3.1 Soil physiochemical properties

The methodology, and instrumentations used for physicochemical properties of soil is shown in table 2

Table 2. Testing items, and methodology for physicochemical properties of soil

| Items                      | Methodology  | Instruments                             |
|----------------------------|--|---|
| Soil texture               | Laser particle analyzer  | APA2000, Marvin company, England        |
| SOC ( $\text{g kg}^{-1}$ ) | $\text{K}_2\text{Cr}_2\text{O}_7$ outside heating              | Semi-micro titrator                     |
| pH                         | 1:5 soil suspension  | pH meter (Inolab WTW series pH 720)     |
| T.N ( $\text{g kg}^{-1}$ ) | NaOH alkaline hydrolysis with reducing agent-diffusion process | Automatic kjeldahl determination method |
| TP ( $\text{g kg}^{-1}$ )  | NaOH liquation   | Spectrophotometer                       |

### 2.3.2 Separation of aggregates and mean weight diameter

The collected undisturbed soil samples were carefully crushed by hand and then passed through 5 mm mesh in the laboratory. Processed air-dried soils of 100 g were kept in the top sieve size of  $> 2$  mm followed by 2-1 mm, 1-0.5 mm, 0.5 -0.25 mm, and  $< 0.25$  mm. All the sieves were immersed in distilled water where it was mechanically shaken with up and down movement for two minutes at 30 cycles per minute. The aggregates obtained from each sieve were oven dried, weighed, and then classified into different size fractions such as  $> 2$  mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm, and  $< 0.25$  mm. These collected air dried WSAs were then analyzed for the SOC, and T.N (Table 2). The isolated fractions of WSAs  $> 0.25$  mm were designated as macro-aggregates and the WSAs  $< 0.25$  mm were considered as micro-aggregates. Calculation for the stability of WSAs was determined by the following equation (1).

$$WSAi (\%) = \frac{Wi}{\text{weight of soil}} * 100 \dots\dots\dots (2)$$

Where,  $\bar{X}_i$  is the mean diameter of aggregates remaining on the respective sieves,  $WSAi$  represent percent mass of aggregates with respect to total weight of soil sample on the  $i$ -th sieve and  $n$  is the sieves number used for the separation of aggregates.

### 2.3.3 Partitioning proportion of the soil organic carbon, and soil total nitrogen

The partitioning proportion of the SOC, and T.N with given aggregate size was computed by the following equation (3).

$$\text{OC in aggregates (\%)} = \text{OCF}_n \times \text{MF}_n / \text{SM} \times 100 \dots \dots \dots (3)$$

Where,  $\text{OCF}_n$  is the concentration of the SOC in aggregate size fraction,  $\text{MF}_n$ , and SM indicates fraction, and unfractionated mass of soil.

#### 2.4 Statistical analysis

The statistical analyses of the data were analyzed with SPSS computer software version 20.0. Significant differences for the treatments mean data were evaluated by  $P < 0.05$  through the least significance difference (LSD) method. Sigma plot 12.5 was used for figures.

### 3. Results

#### 3.1 Distribution and stability of water stable aggregates

The distribution of the aggregate sizes of all the treatments showed largest proportion in WSAs  $< 0.25$  mm (Table 3). The proportion of WSAs  $> 2$  mm in the CK was 3.84 % to 6.89% in the T5 treatment, and is significantly higher than the other treatments ( $P < 0.05$ ). However, no significant differences among Ck, T1, and T2, and between T3, and T4 treatments were found for the WSAs  $> 2$  mm. The WSAs 2-1 mm showed highest proportion of 7.35 % in the T5 followed by T4 treatment in comparison to the other treatments ( $P < 0.05$ ). The range of WSAs 1-0.5 mm in the CK was 4.73 % to 7.61 % in the T5 with highest significant differences were found in comparison to the other treatments ( $P < 0.05$ ). The aggregate size fractions of WSAs 0.5-0.25 mm indicated highest proportion in the T5 treatment than the CK, T1, T2, T3, and T4 treatments ( $P < 0.05$ ). The proportion of WSAs  $< 0.25$  mm were highest in the CK

treatment ( $P < 0.05$ ) and showed significantly a decreasing trend with biochar addition rate specifically in the T5 treatment ( $P < 0.05$ ).

The increasing proportion of the macro aggregates (WSAs  $> 0.25$  mm) and a decreasing proportion of the micro aggregates (WSAs  $< 0.25$  mm) by biochar addition rate (table 3) significantly optimized MWD of the soil aggregates (Figure. 1A). The highest MWD in the T5 indicated highest differences in comparison to the control however, such a difference were lower for the lower rate of biochar addition rate. Therefore suggested that, biochar addition rate in the T5 highly influences the stability of WSAs (MWD).

#### 3.2 Distribution of soil organic carbon

The highest concentration of the SOC ( $\text{g kg}^{-1}$ ) in bulk soil were observed in the in the T5 with a lowest in the control (Figure 1B). Compared to the control, biochar addition rates from T1 to T5 significantly increased SOC concentration. This revealed that biochar addition rate in every treatment significantly influence variations in the SOC concentrations.

The distribution of SOC concentrations in WSAs highly depends on biochar addition rate (Table 4). The concentration of the SOC in the control of WSAs  $> 2$  mm were 4.32  $\text{g kg}^{-1}$  to 5.91  $\text{g kg}^{-1}$  in the T5 treatment ( $P < 0.05$ ). The concentration of the SOC in the T5 was significantly higher than the other treatments ( $P < 0.05$ ) with no significant differences was observed between T3, and T4 treatment. The highest and lowest concentration of SOC in WSAs 2-1 mm was influenced by T5 and CK treatment where T1 and T2 treatment showed no significant differences.

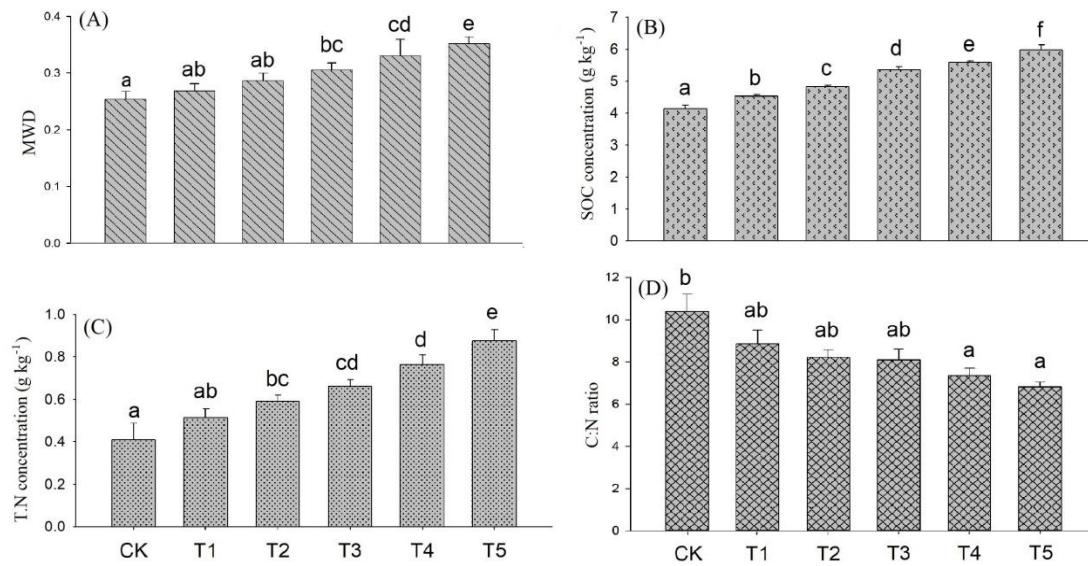


Figure 1. Impact of biochar on soil properties of bulk soil: (A) Mean weight diameter (MWD), (B) Soil organic carbon concentration ( $\text{g kg}^{-1}$ ), (C) Soil total nitrogen concentration ( $\text{g kg}^{-1}$ ), (D) C:N ratio. Note: CK: control; T1:  $4 \text{ t ha}^{-1}$ , T2:  $8 \text{ t ha}^{-1}$ , T3:  $12 \text{ t ha}^{-1}$ , T4:  $16 \text{ t ha}^{-1}$ , T5:  $20 \text{ t ha}^{-1}$ . Different small letters indicate significant differences as determined by the LSD test ( $p \leq 0.05$ ).

**Table 3** Distribution of water stable aggregates (%) under different biochar addition rates.

| Soil aggregates | CK                         | T1                          | T2                          | T3                          | T4                         | T5                         |
|-----------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------|----------------------------|
| > 2 mm          | $4.84 \pm 0.27 \text{ a}$  | $5.15 \pm 0.62 \text{ ab}$  | $5.79 \pm 0.29 \text{ ab}$  | $5.91 \pm 0.62 \text{ abc}$ | $5.95 \pm 0.30 \text{ bc}$ | $6.89 \pm 0.24 \text{ c}$  |
| 2-1 mm          | $4.12 \pm 0.49 \text{ a}$  | $4.73 \pm 0.23 \text{ ab}$  | $5.40 \pm 0.66 \text{ abc}$ | $5.93 \pm 0.12 \text{ abc}$ | $7.02 \pm 0.88 \text{ bc}$ | $7.35 \pm 0.54 \text{ c}$  |
| 1-0.5 mm        | $4.73 \pm 0.19 \text{ a}$  | $5.57 \pm 0.58 \text{ ab}$  | $5.73 \pm 0.60 \text{ ab}$  | $6.01 \pm 0.42 \text{ ab}$  | $6.48 \pm 0.57 \text{ ab}$ | $7.61 \pm 0.25 \text{ b}$  |
| 0.5-0.25 mm     | $3.76 \pm 0.56 \text{ a}$  | $3.20 \pm 0.46 \text{ a}$   | $3.80 \pm 0.40 \text{ a}$   | $5.35 \pm 0.19 \text{ ab}$  | $6.61 \pm 0.60 \text{ b}$  | $7.20 \pm 0.77 \text{ b}$  |
| <0.25 mm        | $82.53 \pm 0.83 \text{ a}$ | $81.34 \pm 1.41 \text{ ab}$ | $81.26 \pm 0.66 \text{ bc}$ | $77.16 \pm 0.88 \text{ cd}$ | $73.93 \pm 1.64 \text{ d}$ | $70.93 \pm 1.13 \text{ d}$ |

Note: CK: control; T1:  $4 \text{ t ha}^{-1}$ , T2:  $8 \text{ t ha}^{-1}$ , T3:  $12 \text{ t ha}^{-1}$ , T4:  $16 \text{ t ha}^{-1}$ , T5:  $20 \text{ t ha}^{-1}$ . Mean value  $\pm$  S.E in the same row followed by the same letter are not significantly different using L.S.D test at  $P < 0.05$  level.

Similarly, the remaining WSAs 1-0.5 mm, 0.5-0.25 mm and < 0.25 mm showed highest SOC concentration with increasing biochar addition rate while no significant difference was found among CK, T1, T2 for WSAs 1-0.5 mm, T1, and T2 for WSAs 0.5-0.25 mm and T1, and T2 as well as T3, T4, and T5 for the WSAs < 0.25 mm treatments respectively.

Partitioning proportion (%) of SOC in WSAs was affected by biochar addition rates (Figure 2). Such a partitioning of SOC with biochar addition rates in WSAs > 0.25 mm showed increasing trend than the control with most of the treatment showed slightly non-significant differences among the T1, T2, T3, and T4 treatment respectively. However, the partitioning proportion (%) of the SOC in WSAs 0.5-0.25 mm was consistently lower than the WSAs > 0.5 mm and WSAs < 0.25mm.

### 3.3 Distribution of total nitrogen

Among all the treatments, biochar addition rate significantly maximized concentration of the total nitrogen in the bulk soil (Figure 1C). The maximum concentration of the T.N was noted in the T5 whereas lowest in the control with no significant difference was observed between CK, and T1, and between T2, and T3 treatments ( $P < 0.05$ ) respectively.

The concentration of the T.N showed increased trend with increasing biochar addition rate (Table 5). Significantly, biochar addition rate in the T5 showed highest T.N concentrations in WSAs > 2 mm than the other treatments ( $P < 0.05$ ). Similarly an increasing trend of T.N was observed for WSAs 2-1 mm with no significant difference was found for CK, T1, and for T3, and T4 with slight significant differences were noted in the T5 treatment. The WSAs 1-0.5 mm showed no significant difference in CK, and T1, slight difference with in the T2 and T3 and for the T4 and T5 treatment ( $P < 0.05$ ).

Table 4. Distribution of the soil organic carbon ( $\text{g kg}^{-1}$ ) under different biochar addition rate.

| Soil aggregates | CK         | T1         | T2         | T3         | T4          | T5         |
|-----------------|------------|------------|------------|------------|-------------|------------|
| > 2 mm          | 4.32±0.08a | 4.51±0.01b | 4.81±0.03c | 5.38±0.03d | 5.46±0.02d  | 5.91±0.02e |
| 2-1 mm          | 4.17±0.06a | 4.47±0.01b | 4.55±0.02b | 5.30±0.03c | 5.45±0.02d  | 5.82±0.02e |
| 1-0.5 mm        | 4.40±0.27a | 4.48±0.02a | 4.56±0.03a | 5.23±0.01b | 5.42±0.03bc | 5.84±0.02c |
| 0.5- 0.25 mm    | 4.12±0.09a | 4.45±0.02b | 4.49±0.02b | 5.27±0.02c | 5.40±0.02cd | 5.52±0.03d |
| < 0.25 mm       | 4.01±0.05a | 4.36±0.03b | 4.50±0.01b | 5.16±0.08c | 5.43±0.01d  | 5.48±0.02d |

Note: CK: control; T1: 4  $\text{t ha}^{-1}$ , T2: 8  $\text{t ha}^{-1}$ , T3: 12  $\text{t ha}^{-1}$ , T4: 16  $\text{t ha}^{-1}$ , T5: 20  $\text{t ha}^{-1}$ . Mean value  $\pm$  S.E in the same row followed by the same letter are not significantly different using L.S.D test at  $P < 0.05$  level.

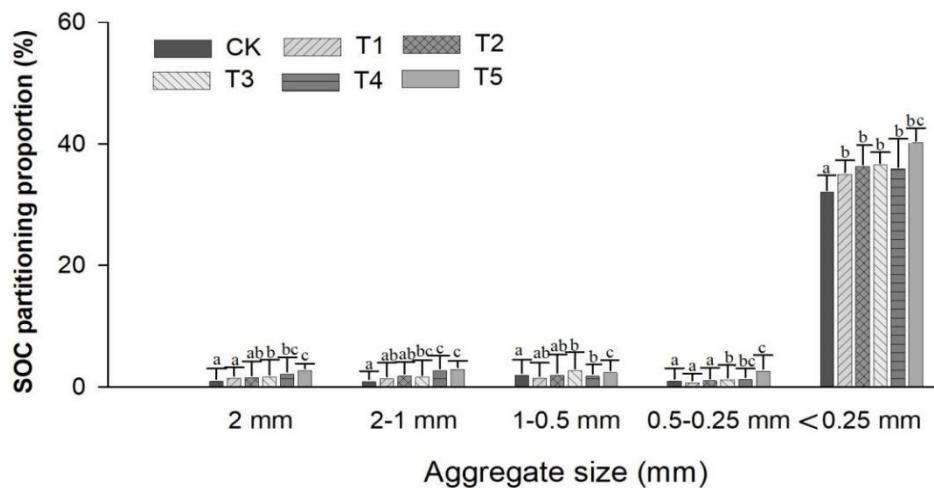


Figure 2. Partitioning proportions (%) of soil organic carbon (SOC) in water stable aggregates as influenced by biochar addition rates. Note: CK: control; T1: 4 t ha<sup>-1</sup>, T2: 8 t ha<sup>-1</sup>, T3: 12 t ha<sup>-1</sup>, T4: 16 t ha<sup>-1</sup>, T5: 20 t ha<sup>-1</sup>. Different small letters indicate significant differences as determined by the LSD test ( $p \leq 0.05$ ).

The highest concentration of the total nitrogen in WSAs 0.5-0.25 and <0.25 mm were 0.66 g kg<sup>-1</sup>, and 0.59 g kg<sup>-1</sup> with a lowest in the control however no significant difference was found among CK, T1, T2 and T3, and with a slight significant differences were observed for the T4, and T5 of WSAs 0.5-0.25 mm, and for CK, T1, and for the T2, T3, and T4 with a slightly significant difference were observed in between the T4, and T5 treatment in the WSAs < 0.25 mm respectively. The partitioning proportion (%) of total nitrogen under different biochar addition rates are presented in Figure 3. The maximum proportions (%) of T.N were observed in WSAs > 0.5 mm under the biochar addition rates except for the T4 in WSAs 1-0.5 mm. The partitioning proportions (%) of T.N in WSAs 0.5-0.25 mm were less than the WSAs > 0.5 mm and

< 0.25 mm. The WSAs < 0.25 mm was affected by biochar addition rate with high partitioning proportion (%) were noted in the T5, however no significant differences were observed among the other treatments

### 3.4 C. N ratio

Biochar addition rate significantly decreased C:N ratio compared to the control of the bulk soil (Figure 1D). Increasing biochar addition rate slightly decrease C:N ratio with no significance differences were noted for the CK, T1, T2, T3, and for the T4, and T5. The lower C:N ratio in the T5 suggest the rapid released of nitrogen into the soil.

The distribution of the C:N ratio within the WSAs was significantly affected by biochar addition rate as shown in Table 6. The highest C:N ratio in the WSAs > 2 mm were observed in the control (9.84 g kg<sup>-1</sup>)

Table 5. Distribution of the soil TN (g kg<sup>-1</sup>) under different rates of biochar.

| Soil aggregates | CK         | T1          | T2           | T3           | T4          | T5         |
|-----------------|------------|-------------|--------------|--------------|-------------|------------|
| > 2 mm          | 0.56±0.01a | 0.60±0.04ab | 0.66±0.04abc | 0.69±0.01abc | 0.71±0.04bc | 0.75±0.02c |
| 2-1 mm          | 0.51±0.02a | 0.54±0.01a  | 0.59±0.03ab  | 0.65±0.03bc  | 0.67±0.01bc | 0.73±0.02c |
| 1-0.5 mm        | 0.48±0.02a | 0.50±0.01ab | 0.56±0.02abc | 0.61±0.02bcd | 0.64±0.01cd | 0.68±0.04d |
| 0.5- 0.25 mm    | 0.45±0.02a | 0.49±0.01ab | 0.52±0.05ab  | 0.56±0.03abc | 0.61±0.02bc | 0.66±0.02c |
| < 0.25 mm       | 0.41±0.01a | 0.44±0.04a  | 0.48±0.03ab  | 0.51±0.03ab  | 0.54±0.01ab | 0.59±0.04b |

CK: control; T1: 4 t ha<sup>-1</sup>, T2: 8t ha<sup>-1</sup>, T3: 12 t ha<sup>-1</sup>, T4: 16 t ha<sup>-1</sup>, T5: 20 t ha<sup>-1</sup>, Mean value ± S.E in the same row followed by the same letter are not significantly different using L.S.D test at  $P<0.05$  level.

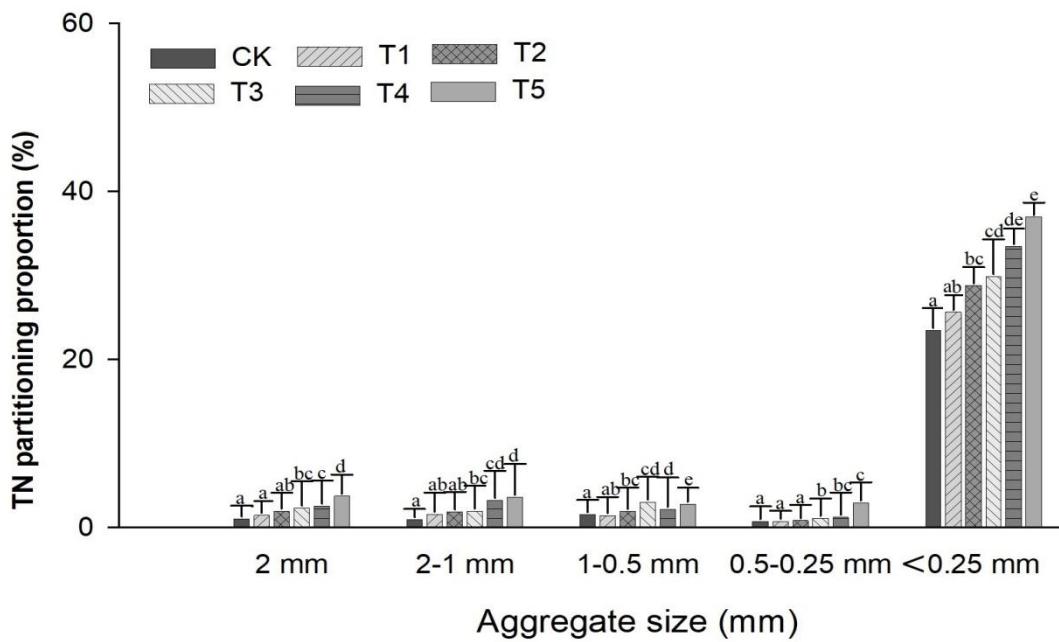


Figure 3. Partitioning proportions (%) of total nitrogen (T.N) in water stable aggregates as influenced by biochar addition rates. Note: CK: control; T1: 4 t ha<sup>-1</sup>, T2: 8t ha<sup>-1</sup>, T3: 12 t ha<sup>-1</sup>, T4: 16 t ha<sup>-1</sup>, T5: 20 t ha<sup>-1</sup>. Different small letters indicate significant differences as determined by the LSD test ( $p \leq 0.05$ ).

Table 6. Distribution of the C:N ratio (g kg<sup>-1</sup>) under different rates of biochar.

| Soil aggregates | CK          | T1           | T2           | T3           | T4          | T5         |
|-----------------|-------------|--------------|--------------|--------------|-------------|------------|
| > 2 mm          | 9.84±0.44c  | 9.12±0.45bc  | 8.31±0.31ab  | 8.09±0.29ab  | 7.42±0.25a  | 6.99±0.23a |
| 2-1 mm          | 10.19±0.52d | 9.80±0.27cd  | 8.75±0.08bc  | 8.47±0.42ab  | 8.06±0.14ab | 7.22±0.15a |
| 1-0.5 mm        | 12.81±1.34c | 11.33±0.35bc | 9.26±0.25ab  | 8.98±0.26ab  | 8.05±0.35a  | 7.83±0.18a |
| 0.5- 0.25 mm    | 11.84±0.73c | 11.83±0.30c  | 10.53±0.24bc | 9.07±0.57ab  | 8.83±0.36ab | 8.37±0.19a |
| < 0.25 mm       | 13.42±1.20b | 13.10±0.27b  | 11.13±0.56ab | 11.03±0.48ab | 9.46±0.46a  | 8.78±0.45a |

Note: CK: control; T1: 4 t ha<sup>-1</sup>, T2: 8t ha<sup>-1</sup>, T3: 12 t ha<sup>-1</sup>, T4: 16 t ha<sup>-1</sup>, T5: 20 t ha<sup>-1</sup>. Mean value ± S.E in the same row followed by the same letter are not significantly different using L.S.D test at  $P < 0.05$  level

with a lowest in T5 (6.99 g kg<sup>-1</sup>), such a ratio relatively showed non-significant differences among the other treatments. The trend of C:N ratio is similar for the other remaining WSAs. Under the different treatments, biochar addition rate especially T5 significantly decreased C:N ratio ranged from 28 %, 29%, 38%, 29%, and 34% for WSAs > 2 mm, 2-1 mm, 1-0.5 mm, 0.5- 0.25 mm, and < 0.25 mm in comparison to the control. However, under the same treatment, C:N ratio showed an increasing trend with decreasing WSAs sizes, such a trend of C:N ratio indicated similar but a decreasing trend with biochar addition rate.

#### 4. Discussion

Although it is considered that biochar addition rates with chemical fertilizers considerably altered the distribution of the soil aggregation. However, such aggregation in the control of our study are similar to the previous finding of who revealed that WSAs are influenced by the dynamics soil

properties. Changes in the dynamic soil properties are limited by the inherent soil or by another dynamic's property. Among the dynamic soil properties, soil aggregation play a key role and showed positive correlation with biochar addition rate (Table 3), which is mainly influenced by the distribution, and availability of organic carbon. Such a soil aggregation acts as a physical barrier for the protection of the organic carbon (Wang et al., 2017), and their stability play a vital role to prevent rapid decomposition (Pulleman & Marinissen, 2004)., In this study, biochar addition rate significantly enhanced soil aggregations in comparison to the control (Table 3) therefore optimized stabilization of the aggregates (Figure 1A). The stability of WSAs in our study could be the hydrophobic bonding of SOC contained in soil aggregates (Piccolo et al., 1996) which therefore acts as a binding agent for soil aggregation and agglomeration of the soil mineral particles in creating aggregates hierarchy (Rabbi et al.,

2020), (Juriga & Šimanský, 2018). Such a stability of the aggregation reduce the disruption of soil structure in sustaining soil cohesion and resistivity to different external environmental disturbances (Rampazzo et al., 1995). The contents of SOC in macro aggregates (Table 4) showed positive correlation with the stability of aggregates (Figure 1. B) in indicating similarity with the previous agreements of (Tisdall & OADES, 1982).

In our study, it has also been found that the highest stability of aggregates with biochar addition rate (T5) might be due to the enhanced fine roots and microbial activities, which assist in binding micro-aggregates, and therefore supporting the formation of soil aggregation as reported by (Deurer et al., 2009). However, the decline MWD of aggregates in the control of our study are similar with the who revealed that fine soil particles could be the dominant attribute in declining the stability, and strength of aggregate. Similarly, (Munkholm et al., 2002) explained that such a soil strength of aggregates showed fragile resistance to destruction under wet condition, consistent to the control of our results. To enhance aggregates resistance to destruction under wet condition, biochar addition rate significantly maximized the proportion of the WSAs  $> 0.25$  mm size fractions, and minimized WSAs  $< 0.25$  mm fraction (Table 3). Our result are similar with (Liu et al., 2014) who reported that water stable aggregates highly depend on biochar addition rate that acts as a conservative role in the protection of the SOC within the macro aggregates. In the same study, biochar addition rate significantly affected the

distribution of SOC within the WSAs, and this possibly might be due to lower rate of SOC in the control which therefore showing similarity with the finding of. However, higher content of soil organic carbon in WSAs, and bulk of the soil (Table 4: Figure 1. B) was attributed to the preferential sequestration of the SOC. Such a sequestration of SOC with biochar addition rate was higher in the WSAs  $> 0.25$  mm than the WSAs  $< 0.25$  mm. Nevertheless, biochar addition rates in the T5 slightly exhibited significant impact on the partitioning proportion of SOC in both the WSAs  $> 0.25$  mm and  $< 0.25$  mm (Figure 2) by changing the proportion of the SOC within aggregates. However, our finding are in opposition with the study of (Tiancong et al., 2005), and (Sodhi et al., 2009) who revealed that long term effect of the organic material enhance partitioning proportion of the SOC in macroaggregates. This might be due to the differences in the inherent and dynamic soil properties such as climatic condition, elevation, organic materials, and soil texture between this and our study.

Aggregate associated T.N followed a similar trend as observed for aggregate associated organic carbon. The combined application of biochar (T5) plus chemical fertilizer resulted in highest proportion of T.N in aggregates compared to the control with prominent effect was observed in WSAs  $> 0.25$  mm size fraction (Table 5). Such a prominent effect of T.N content in WSAs  $> 0.25$  mm might be the microbial activity which are N rich compounds provide binding agents for the aggregation of soil aggregates (Six et al., 2000) therefore, highest N in the macro aggregates rather than

the micro aggregates (Table 4) showing consistency with the previous study of (Onweremadu et al., 2007) which therefore support the theory of hierarchical aggregation (Elliott, 1986). Partitioning of the T.N in aggregates and soil T.N within aggregates followed the same pattern as for the soil organic carbon with biochar addition rates. These finding depicts that biochar addition rates had relatively similar effect on the sequestration and partitioning of the T.N and SOC within the aggregates.

## 5. Conclusion

This study investigated the impact of different rates of biochar (0, 4, 8, 12, 16, and 20 t ha<sup>-1</sup>) in combination with chemical fertilizers (NPK) on water-stable aggregates (WSAs) and their association with soil organic carbon (SOC), total nitrogen (T.N), mean weight diameter (MWD), carbon-to-nitrogen (C: N) ratio, and partitioning proportion (%) of SOC and T.N in an apple orchard soil. The results revealed that the rate of biochar addition played a crucial role in influencing all the studied parameters. Among the different biochar rates, the addition of 20 t ha<sup>-1</sup> (T5) significantly improved the distribution of WSAs, MWD, SOC, and T.N within the aggregates, showing a notable response. However, the C:N ratio exhibited an opposite trend compared to the control. Furthermore, the addition of biochar at the T5 rate significantly enhanced the partitioning proportions (%) of SOC and T.N in the macro-aggregates, although these proportions were lower than those in the micro-aggregates. Overall, this study highlights the potential of using biochar at the T5 rate, along with chemical fertilizers, to improve

the distribution and stability of WSAs, as well as the associated SOC and T.N. However, further research is required to assess the impact on apple quality and productivity, promoting a sustainable farming system.

## Author Contribution

AS and JB, conceptualized and designed the research work. AS conducted the experiment, data analysis and wrote the first draft; JB revised the manuscript and give suggestions. Both authors authors edited the manuscript, read and approved the final version.

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## Conflicts of Interest

The authors declare no conflict of interest.

## Availability of Data and Materials

Data will be available on formal request from the corresponding authors.

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